imaging and multi-color imaging. Here we report the fusion properties of several commonly used RFPs by using an environment sensitive membrane protein Orai1 and reveal that intracellular artificial puncta are actually colocalized with lysosome, indicating that besides monomeric properties, pKa value of RFP is also a key factor for correct localization. In summary, our current study established a fast and easy method to evaluate RFPs fusion properties and provides a useful guide for choosing appropriate RFP for labeling secretory membrane proteins. Among RFPs tested, mOrange2 is highly recommended based on excellent monomeric property, appropriate pKa and high brightness.

1619-Pos Board B570
"Decorating" Cells with Genetically Encoded Fluorescent Proteins - What Color Suits Your Experiment Best?
Li-Chun Tu, Juahdi Monbo, Aviva Joseph, David Grunwald.
RNA Therapeutics Institute, UMass Medical school, Worcester, MA, USA.
A key aspect of simultaneous multicolor single molecule real-time (SMRT) imaging in the living cell is choosing the appropriate combinations of fluorescent proteins (FPs). Although ample information is available on fluorescent brightness, photo-stability, pH sensitivity and natural oligomerization state of FPs, interpolating published data into performance in live human cell experiments is difficult. Ultimately the effort of re-cloning of genetic constructs and advanced quantitative analysis to determine the most suitable FP favor the use of suboptimal label combinations. However, the use of high power to excite fluoresphores often results in shorter observation times, higher photo-damage and possibly the induction of 'stress' reaction by the cell. To determine the best FP combination for multicolor imaging relies on not only obvious factors like optimal spectral separation, best photostability and highest brightness but also the amount of excitation power needed to generate sufficient signal at minimal background noise level which could come from live cell autofluorescence, cross-talk between different channels and cross-excitation of FP.
We tested 36 different FPs in a parameter space specific to their suitability in live cell multicolor single molecule imaging. Particular attention was paid to biased subcellular localization effects and performance of the FPs for labeling of dynamic (soluble FP & diffusive fusion protein), labeling of confined molecules (nuclear pore marker on nuclear envelope), the amount of fluorescence signal under low excitation power imaging conditions and cross excitation and cross emission at non-maximal photon output.
We present different sets of FPs for different labeling purposes and discuss possible protocols for testing and specifying FP performance in low light live cell imaging conditions.

1620-Pos Board B571
Confocal Absorption Microscopy of Biomolecules in the Atto-Mole Range
Alfons Schulte[1], Fatholah Salehi[2], Michael Sigman[3].
[1]Physics and College of Optics, University of Central Florida, Orlando, FL, USA, [2]Physics, University of Central Florida, Orlando, FL, USA, [3]Chemistry and College of Optics, University of Central Florida, Orlando, FL, USA.
A versatile approach for absorption spectroscopy on the micron scale is presented allowing label-free characterization of as few as 10^7 molecules in a pico-liter volume. Our setup employs a confocal microscope with spatial resolution at the cellular level (1-2 microns) over a wide spectral range from the near-infrared to the ultraviolet. Spectra of amino acid, proteins, and DNA bases in solution are acquired on a timescale of seconds with spectral resolution of 0.3 nm. We analyze samples in nanoliter quantities containing a few concentrations of two different dyes. In combinations with factor analysis we can reconstruct the spectra as a superposition of the individual dyes. We explore applications to spatially inhomogeneous samples and single cells.

1621-Pos Board B572
Testing a Diffusion Trap Model for Store-Operated Calcium Entry by Single Particle Tracking
Minnie M. Wu, Elizabeth D. Covington, Richard S. Lewis.
Molecular & Cellular Physiology, Stanford University School of Medicine, Stanford, CA, USA.
Depletion of calcium from the endoplasmic reticulum (ER) triggers autonomous assembly of STIM1-Orai1 complexes at ER-plasma membrane (PM) junctions, leading to store-operated calcium entry (SOCE). One hypothesis to explain this process is a diffusion trap in which activated STIM1 diffusing in the ER becomes trapped at junctions by binding to the PM via its C-terminal polybasic domain. STIM1 then traps Orai1 in the PM through binding of STIM1's CRAC activation domain. We tested this diffusion trap model by analyzing STIM1 and Orai1 diffusion using single-particle tracking, photoactivation of protein ensembles, and Monte Carlo simulations. In resting cells, STIM1 diffusion is Brownian with a mean diffusion coefficient (D) of 0.12 $\mu m^2/s$, while Orai1 is slightly subdiffusive (mean D=0.09 $\mu m^2/s$). After store depletion both proteins slow to the same speed (mean D≈0.03 $\mu m^2/s$), consistent with complex formation, and are confined to an area similar in size to ER-PM junctions. At high STIM1:Orai1 expression ratios, the probability that Orai1 escapes the diffusion trap is >1%, but the escape probability is significantly increased by reducing the affinity of STIM1 for Orai1 or by expressing the two proteins at comparable levels. Our results provide direct evidence that STIM1 and Orai1 are trapped by their physical connections across the junctional gap. However, we also find that STIM-Orai interactions are surprisingly dynamic in that readily reversible binding reactions generate free STIM1 and Orai1 which can escape and exchange with extrajunctional pools. These data suggest that STIM1 and Orai1 interact with low affinity, creating the potential for SOCE modulation even when ER calcium stores are mostly depleted, and promoting the reversal of SOCE upon store refilling.

1622-Pos Board B573
Membrane-Protein Diffusion in E. coli: A Random Walk in a Heterogeneous Landscape
Aravindan Varadarajan[1,2], Felix Oswald[1,3], Yves J.M. Bollen[2,3], Erwin J.G. Peterman[1,2].
[1]Department of Physics and Astronomy, VU University, Amsterdam, Netherlands, [2]LaserLab Amsterdam, VU University, Amsterdam, Netherlands, [3]Department of Molecular Cell Biology, VU University, Amsterdam, Netherlands.
Membrane proteins perform vital cellular functions like respiration, signaling and nutrient uptake. For proper membrane-protein functioning, conformational dynamics, complex formation and ability to diffuse in the membrane are vital parameters. Despite a lot of work on model membranes, little is known about lateral diffusion of proteins in prokaryotic membranes. Here we use single-molecule wide-field epi-fluorescence microscopy to track, in living E. coli, the lateral mobility of seven trans-membrane proteins of different size fused to green fluorescent protein. We apply a novel method, IPODD (inverse projection of displacement distributions), to extract accurate diffusion coefficients from the 2-D projected diffusion trajectories along the 3-D curved bacterial membrane. The diffusion coefficients we find are significantly lower than those reported in in vitro studies of isolated membrane proteins in giant unilaminar vesicles. Our results indicate that crowding in the E. coli inner membrane substantially slows down trans-membrane protein mobility. Strikingly, we observe heterogeneity in the diffusive motion of all seven proteins: they all show a faster and a slower moving component. This heterogeneity does not appear to be connected to specific localization of the proteins in poles or other parts of the bacterium. Instead, our experiments indicate that the plasma membrane of E. coli contains patches with a different lipid composition than the bulk of the membrane, resulting in regions of slower and faster membrane-protein diffusion. These results show that the diffusion behavior of proteins embedded in the plasma membrane in E. coli is richer and far more complex than anticipated.

1623-Pos Board B574
Single-Molecule Study of RelA Diffusion in Live E. coli Cells during the Stringent Response
Wenting Li[1], Heejun Choi[1], Yan Zhang[2], Emmanuelle Bouveret[3], James C. Weisshaar[1].
[1]Department of Chemistry, University of Wisconsin-Madison, Madison, WI, USA, [2]Department of Bacteriology, University of Wisconsin-Madison, Madison, WI, USA, [3]Aix-Marseille University, France.
The stringent response is a physiological response that occurs when bacterial cells encounter nutritional stresses such as amino acid starvation or fatty acid starvation. The most marked outcome of this response is an immediate accumulation of the effector nucleotides, guanosine tetra- and pentaphosphate (ppGpp and pppGpp).The RelA protein of *Escherichia coli* is a (p)ppGpp synthetase that is activated by amino acid starvation. Here, we use single-molecule tracking to investigate the RelA protein diffusive behavior and association with ribosomes both before and after the stringent response. In earlier work from the Elf lab, a RelA-Dendra2 construct diffused like ribosomes (slowly) in normal growth conditions and diffused freely (rapidly) following the stringent response. In sharp contrast, we find that diffusion of a RelA-eYFP construct is heterogeneous in both conditions. Diffusion is much slower under stringent conditions. The single-molecule diffusive behavior is well fit by a hidden Markov model including two diffusive states: free and ribosome-bound. Amino acid starvation increases the dwell time of RelA in the state with lower mobility (ribosome-like diffusion) from 15 ms to 95 ms, while the dwell time of RelA spent in the free state remains unchanged at 20 ms. Correspondingly, the fraction of ribosome-bound RelA increases from 43% to 83% upon amino acid starvation. These observations suggest that RelA binds to ribosomes during synthesis of (p)ppGpp.